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PATENT

Attorney Reference Number 6947-67205-01
Application Number 08/776,350

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Brown *et al.*
Application No. 08/776,350
Filed: April 18, 1997
Confirmation No. 1818
For: TREATMENT OF CANCER USING HSV MUTANT
Examiner: Susan Ungar, Ph.D.
Art Unit: 1642
Attorney Reference No. 6947-67205-01

DECLARATION OF S. MOIRA BROWN, PH.D., FRCPath, FRSE
(Pursuant to 37 C.F.R. § 1.132)

I, S. Moira Brown, Ph.D. FRCPath, FRSE, hereby declare as follows:

(1) I am Chief Scientist and Director of Crusade Laboratories Ltd and Honorary Senior Research Fellow and Professor Emeritus, at The University of Glasgow, Division of Clinical Neurosciences, Institute of Neurological Sciences, Southern General Hospital NHS Trust, Glasgow, G51 4TF, United Kingdom;

(2) I am an inventor on United States patent application 08/776,350.

(3) I am an expert in the field of herpes simplex virus having carried out research into herpes simplex virus from 1968 to the present time. Much of my research in recent years has involved investigation of the potential uses of certain herpes simplex viruses in the treatment of various tumor types. My status as an expert in this field is evidenced by my *curriculum vitae*, a copy of which is attached to this declaration.

(4) In connection with United States patent application 08/776,350 I understand that it is important for the examiner to have an understanding of (i) the differences between primary

and secondary tumors; and (ii) the different replicative properties and modes of replication exhibited by herpes simplex virus *in vitro* and *in vivo*.

(5) Primary and Secondary Tumors

(5.1) Primary tumors are tumors which arise within a single organ and have the pathology of that organ.

(5.2) As an example, in the brain the most common primary tumors are those of astrocytic origin. Astrocytes are cells which are located within the brain and are not present in any other organ in the body. Primary tumors of astrocytic origin often arise when there is a mutation(s) within an astrocyte which leads to cellular transformation and tumor formation. All the cells within a primary astrocytic tumor are then astrocytic in origin.

(5.3) Secondary tumors or metastatic tumors are tumors which have arisen in one organ but have disseminated to other organs which are totally unrelated. For example a mammary tumor of the breast may frequently metastasise to other organs including lung, liver and brain. The tumor cells within these secondary tumors remain mammary carcinoma cells. They do not become lung, liver or brain cells. Their lineage remains unchanged irrespective of the fact that they are not in the organ of origin.

(5.4) The behavior of a secondary tumor in the unrelated organ is different to the behavior in the organ of origin. For example a tumor of the breast within the breast is exposed to the normal factors present in the breast, e.g. specific hormones, whereas in the brain there is no exposure of the mammary carcinoma cells to these hormones and subsequently factors such as the rate of growth and the cell cycle pattern of the tumor can be quite different depending on whether the tumor cells are situated in the breast or in the brain.

(5.5) Following from this, it does not follow that a treatment which is effective for a primary tumor will necessarily be effective for a secondary tumor derived from that primary tumor. This may be true even in the case where the pathology of the primary and secondary

tumors are the same. For example a mammary tumor within the breast may respond to a therapeutic agent which operates by a mechanism targeted at hormonal control whereas in the brain, where hormonal factors are not relevant, the same treatment may have no effect. Additionally although primary tumors may respond to chemotherapy, secondary tumors of any origin which occur in the brain are less amenable to any systemic therapy due to the impermeability of the blood brain barrier.

(5.6) It should also be noted that a secondary tumor does not equate with a recurrent tumor. A recurrent tumor is classified as the recurrence of a tumor within the primary organ. For example within the brain it is common, following surgery to remove a primary tumor, that there will be a recurrence of that tumor within the brain with no dissemination to form secondary tumors outwith the brain.

(6) Herpes Simplex Virus Replication

(6.1) Infection may be said to be the ability of a herpes simplex virus to adsorb onto a cell and enter that cell. This is necessary before replication can occur but is a distinct process to replication.

(6.2) Replication may generally be said to involve replication of viral DNA, the production of virus proteins (usually of all categories i.e. immediate early, early and late), and the assembly of those proteins and replicated DNA into virus particles for the production of progeny virus able to infect surrounding cells. Thus, the result of replication is an increase in the number of potentially infectious virus particles.

(6.3) There can be a full replication cycle as described in 6.2 above and this full cycle of replication may lead to cell death due to lysis or bursting of the cell by the progeny virus.

Alternatively, there can be a part replication cycle involving a stop at any stage in this process. For example, after infection, there can be DNA replication and production of immediate early viral proteins and then a block. In fact, the block can take place at any point

along the replication process. In some cells certain herpes simplex viruses go through the replication cycle to the point of production of progeny virus but a block prevents release of the infectious virus particles from the cell. The virus has still been replicated but it is not released by lysis of the cell.

The spread of herpes simplex virus infection within the human or animal body is not dependent on lysis. Herpes simplex virus can spread by cell fusion of infected and uninfected cells and by the formation of syncitia. No cell lysis occurs in these processes.

The term "replication'" is therefore somewhat generic and can cover a number of situations involving the full or 'lytic' replication cycle or a part or 'non-lytic' replication cycle. In the latter it is perfectly possible for infectious virus particles to be 'replicated' but the process does not have to end in lysis of the infected cell.

(6.4) Latency is a term specifically applied to the situation following HSV infection of neurons of the peripheral nervous system. In these cells, the virus does not go through a full replication cycle but goes into a state where there is only transcription from one region of the genome, the LAT (latency associated transcript), there is no virus protein production and the virus is undetectable apart from the LAT. However it can reactivate from this dormant state and can go into a replication cycle which can be a full cycle or a part cycle. That is, on reactivation replication can go through to lysis or to production of infectious progeny which are not released from the cell, i.e. non-lytic replication.

(6.5) Virulence is a term applied to infection *in vivo* and would normally be considered as the ability to replicate and cause disease, specifically encephalitis. Disease will only occur where there has been rampant, uncontrolled complete cycles of replication, cell death and organ destruction. It is possible for viruses to replicate yet not be considered virulent because the replication cycle has been blocked at a stage before the virus is able to cause cell death and disease.

(6.6) Cells grown in tissue culture (i.e. *in* vitro) go through the cell cycle and replicate. They can be put into growth arrest by using cell cycle blocking chemicals, by serum deprivation or in some cell types by contact inhibition induced when they come in contact with each other.

The ability of a virus to replicate in cells *in vitro* cannot be equated to the ability of that virus to replicate in the same cells *in vivo*. *In vitro* cells can be made amenable to replication of herpes simplex virus by controlling the environment in which they are grown e.g. by the constituents of the growth medium. This is done in order to enable lytic replication such that the plaques formed by a lytically replicating virus can be visualized and picked for further purification and study. It is also done in order to quantitate and titrate infectious virus. *In vivo* this is not the case; cells of a given tissue are in their normal physiological surrounding, they may be dividing cells progressing through the cell cycle, they may be arrested cells not normally dividing and requiring a stimulus to overcome the cell cycle arrest or they may be terminally differentiated and not capable of further division. Some cells will be necrotic and dead.

The result is that lytic replication in a specific cell type *in vitro* does not necessarily mean that this will happen *in vivo* such that *in vivo* it is not possible to expect a particular herpes simplex virus to exhibit the same replication profile observed *in vitro*.

(6.7) From the above, one can see that replication does not necessarily lead to virulence. About 80% of the world's population have a latent herpes simplex virus infection. This involves replication of the virus in order to maintain the infection but this is not necessarily lytic or virulent. Indeed, if it were virulent in most cases that would cause the death of the individual and it would not be possible for that 80% of the population to survive and maintain the latent infection.

(7.) All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such

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willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 6th October '04

By: S. Moira Brown
S. Moira Brown, Ph.D, FRCPath, FRSE

CURRICULUM VITAE

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DATE OF BIRTH: 21st March 1946

MARITAL STATUS: Married 1970, Alasdair MacDougall Brown

NATIONALITY: British

EDUCATION & QUALIFICATIONS:

1951-1964	Greenock Academy, Greenock, Scotland Strathearn School, Belfast, N. Ireland
1964-1968	Queen's University, Belfast BSc (Hons IIi), Microbiology
1968-1971	Glasgow University PhD Virology
1989	MRCPath, Royal College of Pathologists
1997	FRCPath, Royal College of Pathologists
1999	FRSE, Royal Society of Edinburgh

EMPLOYMENT:

1971-1995(Dec.)	MRC Virology Unit, Glasgow. MRC appointments to Senior Scientist grade
1977-1980	Invited guest scientist; The Wistar institute, Philadelphia
1996(Jan.) - 2003	Professor of Neurovirology, University of Glasgow
2000 -	Director and Chief Scientist, Crusade Laboratories Ltd.

2003- Professor Emeritus and Honorary Senior Research Fellow, University of Glasgow

PUBLICATIONS: See attached list

RESEARCH FUNDING: 1990 -2004

Medical Research Council 1972-1992 Virology Unit **core funding**
Herpes simplex virus genetics and pathogenesis

The Wellcome Trust 1990-1991 **£30,00** Analysis of HSV mutants

The Wellcome Trust 1991-1992 **£25,000** Neurovirulence studies on HSV

Equine Virology Research Foundation(EVRF) 1992-1994 **£67,000** Mutation of the 5 genes unique to equine herpes virus type 1

EVRF 1994-199 **£240,405** Construction of EHV1 deletion mutants for assessment as vaccine candidates

SmithKline Beecham 1989-199 **£400,000** Development of human herpes virus vaccines

Parkinson's Disease Society 1993-1995 **£111,800** Gene therapy for Parkinson's Disease

The Wellcome Trust 1993-1995 **£135,869** Gene therapy for neurological diseases

Jules Thorn Trust 1994-1996 **£125,000** Gene therapy for recurrent herpes virus infections

BUPA Foundation 1995 **£17,000** Generation of coliphage antibodies against HSV

Medical Research Council 1992-1996 £480,000 The herpes simplex virus neurovirulence factor ICP34.5: its role *in vivo* and *in vitro*

Medical Research Council 1996-1999 **£287,000** The function of the herpes simplex virus neurovirulence factor ICP34.5 and its interaction with cellular proteins

Medical Research Council 1997-2000 **£171,944** Phase 1 study of intra-tumoural injection with the ICP34.5 negative HSV mutant 1716 in patients with malignant glioma (R.Rampling, **S.M. Brown,** G.Cruickshank)

The Scottish Office, Dept. of Health (BTRC) 1998-2000 **£126,000** A pilot study of dose requirements, safety and efficacy of intratumoural injection into secondary melanoma of genetically modified non virulent herpes simplex virus (ICP34.5 negative HSV mutant 1716) (R.MacKie, **S.M. Brown)**

Neurosciences Foundation 1997-2001 **£100,000** Development of HSV for CNS cancer therapy

Medical Research Council 1999-2004 **Cooperative Group Grant** 'Brain Injury and Disease - from mechanisms to man' (J. McCulloch, **SM Brown**, IM Macrae, G. Teasdale)

Biotechnology and Biological Sciences Research Council 2000-2002 **£83,708** Expression, purification and crystallisation of the herpes simplex virus virulence factor ICP34.5. (A. Freer, **SM Brown)**

Scottish Hospital Endowments Research Trust (SHERT) Oct. 2000-2002 **£69,847** Assessment *in vitro* of a new treatment for glioma combining virally mediated cell lysis with gene transfer and targeted radiotherapy. (M.Boyd, **S.M.Brown**, R.J.Mairs and W.J. Argerson.)

The Scottish Executive Department of Health (BTRC) Oct. 2000- 2002 **£120,000** A laboratory and clinical study of the synergy between radiation and the herpes simplex virus mutant HSV1716 as a treatment for brain tumours. (R. Rampling, **S.M. Brown**, R.J. Mairs, M. Boyd and V. Papanastassiou)

The Scottish Executive, Scottish Enterprise, Proof of Concept Fund April 2001 – April 2003 **£199.000** Novel targets for therapeutic intervention in stroke and neurodegenerative diseases. (**S. M. Brown**, J. Harland, I.M. Macrae and J. McCulloch). Oct. 2001- Oct. 2003 **£113,000**

The Scottish Executive, Scottish Enterprise, SMART award. 1999-2000 **£45,000** Novel virus vectors for cancer therapy

The Scottish Executive, Scottish Enterprise, SCIS award. 1999-2000 **£45,000** Diagnostic technology.

The Scottish Executive, Chief Scientist Office, BTRC Jan.2002 – Feb. 2003 **£72,060** Trial of Preoperative Intratumoural Injection with HSV1716 in Patients with Resectable Squamous Cell Tumours of the Head and Neck (**SM. Brown**, D. Soutar, R. Rampling and I. Ganly)

JIF/SHEFC RDG 2001 **£2.27M** Small bore MRI scanner for Glasgow University (J. McCulloch, IR Griffiths, JAR. Nicoll, IM. Macrae, BR. Condon, **SM. Brown** and A. Williams).

The Scottish Executive, Chief Scientist Office, BTRC April 2002 – April 2003 **£48,685** Development of a SPECT tracer to image the distribution of the oncolytic herpes simplex virus HSV1716 in the treatment of glioma and other cancers. (D. Wyper, J. Owens, **S. M. Brown**, J. Patterson, V. Papanastassiou, R. Rampling, D. Hadley, A.T. Elliott)

The Scottish Executive, Scottish Enterprise, SMART award. 2003-2005 **£153,814** Development of combination oncloytic HSV therapy with prodrug therapy.

Dayspring Ventures Core funding for Crusade Laboratories >>£M

RELEVANT PROFESSIONAL INFORMATION:

- Founder, Director and Chief Scientist, Crusade Laboratories Ltd. Glasgow www.crusadelabs.co.uk
- Professor Emeritus and Honorary Senior Research Fellow, University of Glasgow
- Chairman, Scottish Hospital Endowments Research Trust (SHERT) – a non departmental public body (NDPB) and Scotland's largest healthcare charity www.shert.org
- Member Biomedical and Therapeutics Research Committee, Scottish Executive, Health Dept., Chief Scientist Office (Until Jan 2002 when appointed as Chairman of SHERT).
- Convenor, The Royal Society of Edinburgh, Sectional Committee – 'Cell and Molecular Biology'

- Member Glasgow University, Home Office Licence Applications Panel 1999-2002
- Member Glasgow University, Home Office Ethical Review Panel 1999-2002
- Member of the Advisory Board of The Neuroscience Foundation 1997-2000
- Speaker and chairperson at national and international meetings eg International Virology Congresses; Herpesvirus Workshops; New York Academy of Sciences; MS Society of US; WHO meetings; International Society of Neurosurgeons; Royal Society of Medicine; Dept. of Health, GTAC.
- Referee for Programme and Project grant applications, for Senior Fellowships and for Review Boards: MRC; Wellcome Trust; NERC; BBSRC; Parkinson's Disease Society; Horse Race Betting Levy Board; Irish Health Board; Israeli Science Foundation; Royal College of Surgeons.
- Referee for professional journals
- Ph D student supervision - 21 since 1985 - all successfully completed
- External examiner University of Cambridge and University of Birmingham
- Chosen to exhibit at The Royal Society Summer Exhibition July 2001. 'Killer to Cure – herpes simplex virus in cancer therapy' 21 exhibits chosen from the UK. **http://www.sc1.ac.uk/discover/kill.cfm**
- Invited to feature personally on The Royal Society website **http://www.sc1.ac.uk/meet/moibo.cfm** 'What is science and why does it matter? Visit this site to access some of the most important, exciting, and respected knowledge in the world' Sub section 'Meet Moira Brown'
- Invited academic in a group of 9 business and government officials on a 7 day UK Dept. of Trade and Industry fact-finding mission on Oncology to the West Coast USA, in June 2001.
- My work featured in the BBC1 documentary series 'Superhuman', presented by Lord Winston in Nov. 2000, in the episode 'Killer to Cure'
- Invited to feature personally in The Lancet, 'Lifeline' column July 14th 2001
- Member, The Athenaeum, London

PATENT FAMILIES:

Filed and published and in various stages of prosecution. I am the principal inventor.

1. HSV mutant 1716; use in vaccines 1991 PCT/GB92/00179. Granted, UK, USA and world wide
2. HSV mutant 1716; use in CNS cancer therapy 1994 PCT/GB95/01791 Granted USA, UK and world wide
3. HSV mutant 1716; use in non-CNS cancer therapy 1996 9603872.4 Granted USA ,UK and world wide
4. HSV mutants 1716 and 1764; use as gene therapy vectors 1996 9615794.6 Granted USA, UK and worldwide
5. Methods for identifying cell cycle regulators 1997 PCT/GB98/00772 Granted USA, UK and world wide

6. Novel targets for therapeutic intervention in stroke. Filed July 2000
7. Novel targets for identifying cell cycle regulators Filed Aug. 2000 Granted 2002
8. Novel application of HSV1716 in dermatological conditions Filed March 2001
9. Novel HSV complex Filed Jan. 2002 Granted Mar. 2003
10. Methods for generating mutant viruses Filed Dec. 2003
11. Novel herpes simplex viruses Filed April 2004
12. EHV mutant ED71; use as novel vaccine 1996. Granted Licensed to Intervet, 1997
13. EHV mutant ED75; use as novel vaccine 1996 Granted
14. EHV mutant ED1; novel vaccine 1996 Granted

COMMERCIAL

The HSV patent families listed above are owned by GU and those relating to HSV have been licensed to a biotech company 'Crusade Laboratories Ltd.' I am the founder of this company which is owned by Dayspring Ventures Ltd, Glasgow University and Cancer Research Ventures (CRUK). The company is focused on developing selectively replication competent HSV for cancer therapy and on biological therapies for neurodegenerative diseases. We have completed Phase 1 trials for our prototype product HSV1716 in cancer and are about to enter efficacy trials in Europe in glioma patients. I am the Chief Scientific Officer (CSO) and a director of the company. www.crusadelabs.co.uk

INTERESTS: Reading; music; contemporary Scottish painting; Christian faith; dogs; walking and travel.

RESUME:

Chief Scientist and director of Crusade Laboratories Ltd.

University research professor

Chairman of The Scottish Hospital Endowments Research Trust (SHERT)

Convenor of the Royal Society of Edinburgh Sectional Committee 'Cell and Molecular Biology'

Experienced in obtaining grant funding from the public and private sector

Expertise in Scottish Enterprise grant funding aimed at commercialisation – SMART, SCIS, POC

Inventor of intellectual property

Committee member of grant funding bodies and the Royal Society of Edinburgh

Pioneer in the translation of research findings from the 'bench to the patient'

Team leader in negotiations with regulatory bodies involved in 'gene therapy' i.e. HSE, GTAC, MCA and EMEA

Communicator to the cognoscente, the 'man in the street', the business community and the media

Able to act at the interface between scientists and clinicians

Excellent leadership skills and very much a team player

I am an active, well-established research scientist with over 30 years experience in the field of molecular virology. I run a research group and a company with an international reputation and have pioneered the use of oncolytic herpes simplex virus in the treatment of cancer. I have a very strong commitment to translating ideas from the laboratory into realistic therapeutic advances in medicine and have been instrumental in showing that basic academic discoveries can be translated to patient therapy and to commercial enterprise.

I am a highly organised and focused person who has the ability to be multifunctional. I have a strong personal commitment to the work ethic and adopt a totally straightforward and honest approach to my work and to my dealings with others. I have the skills to lead a team, to listen and take note of the opinions of others and to take responsibility for decision-making.

PUBLICATIONS OF PROFESSOR S M BROWN

1. BROWN, S.M., RITCHIE, D.A. and SUBAK-SHARPE, J.H (1973). Genetic studies with herpes simplex virus type 1. The isolation of temperature sensitive mutants, their ordering into complementation groups and recombination analysis leading to a linkage map. *Journal of General Virology* 18, 329-346.

2. SUBAK-SHARPE, J.H., BROWN, S.M., RITCHIE, D.A., TIMBURY, M.C. and HALLIBURTON, I.W. (1973). Herpes virus genetics. Schering Workshop on Virus/Cell Interactions. Advanced Bioscience II, 205-218. Pergamon Press/Vieweg. Braunschweig, New York, Oxford.

3. BROWN, S.M. and RITCHIE, D.A. (1975). Genetic studies with herpes simplex virus type 1. Analysis of mixed plaque forming virus and its bearing on genetic recombination. *Virology* 64, 32-42.

4. BROWN, S.M. and RITCHIE, D.A. (1975). Genetic studies with herpes simplex virus type 1. Quantitative analysis of the products from two-factor crosses. *Virology* 64, 281-283.

5. SUBAK-SHARPE, J.H., BROWN, S.M., RITCHIE, D.A., TIMBURY, M.C., MACNAB, J.C.M., MARSDEN, H.S. and HAY, J. (1975). Genetical and biochemical studies with herpes virus. *Cold Spring Harbor Symposium* 39, 717-730.

6. RITCHIE, D.A., BROWN, S.M., SUBAK-SHARPE, J.H. and JAMIESON, A.T. (1977). Heterozygosis and genetic recombination in herpes simplex virus type 1 virus. *Virology* 82, 323-333.

7. HAY, J., BROWN, S.M., JAMIESON, A.T., RIXON, F.J., MOSS, H., DARGAN, D. and SUBAK-SHARPE, J.H. (1977). The effect of phosphonoacetic acid on herpes viruses. *Journal of Antimicrobial Chemotherapy*, Supp A, 63-70.

8. WARREN, K.G., DEVLIN, M., GILDEN, D.H., WROBLEWSKA, A., BROWN, S.M. and SUBAK-SHARPE, J.H. (1977). Isolation of herpes simplex virus from human trigeminal ganglia, including ganglia from one patient with multiple sclerosis. *Lancet* II, 637-639.

9. WARREN, K.G., BROWN, S.M., WROBLEWSKA, Z., GILDEN, D.H., KOPROWSKI, H. and SUBAK-SHARPE, J.H. (1978). Isolation of latent herpes simplex virus from the superior cervical and vagus ganglia of humans. *New England Journal of Medicine* 298, 1068-1069.

10. WARREN, K.G., WROBLEWSKA, Z., OKABE, H, BROWN, S.M., GILDEN, D.H., KOPROWSKI, H., RORKE, L.B., SUBAK-SHARPE, J.H. and YONEZAWA, I. (1978). Virology and histopathology of the trigeminal ganglia of Americans and Japanese. *J. Can. Sci. Neurol.* 5, 425-430.

11. BONE, D.R., BROWN, S.M., CROMBIE, I. and FRANCKE, B. (1978). Viral DNA synthesis in cells infected with temperature sensitive mutants of herpes simplex virus type 1. *Journal of Virology* 28, 14-20.

12. BROWN, S.M., SUBAK-SHARPE, J.H., WARREN, K.G., WROBLEWSKA, Z and KOPROWSKI, H (1979). Detection of defective or uninducible herpes simplex virus genomes latent in human ganglion explants. *Proceedings of the National Academy of Sciences, USA* 76, 2364-2368.

13. LONSDALE, D.M., BROWN, S.M., SUBAK-SHARPE, J.H., WARREN, K.G. and KOPROWSKI, H. (1979). The polypeptide and the DNA restriction enzyme profiles of spontaneous isolates of herpes simplex virus type 1 explants of human trigeminal, superior cervical and vagus ganglia. *Journal of General Virology* 43, 151-171.

14. LONSDALE, D.M., BROWN, S.M., LANG, J., SUBAK-SHARPE, J.H., KOPROWSKI, H. and WARREN, K. (1980). Variations in HSV isolated from human ganglia and the study on clonal variations in HSV-1. 'Genetic Variations of Viruses'. *Ann New York Acad Sci* 354, 291-308.

15. BROWN, S.M. and STEVENS, J. (1981). Latency of herpesviruses. In: 'The Human Herpesviruses, An Interdisciplinary Perspective', (eds) A.J. Nahmias, W.R. Dowdle and R.F. Schinazi. Elsevier North Holland Inc.

16. LEWIS, M.E., WARREN, K.G., QUINN, J., BROWN, S.M., SUBAK-SHARPE, J.H., PATY, D.W., YOUNG, B. ZHIBTNER, A. and Kettlys, G.D. (1981). Comparison of herpesvirus encephalitis isolates by restriction enzyme analysis. In: 'The Human Herpesviruses, An Interdisciplinary Perspective', (eds) A.J. Nahmias, W.R. Dowdle and R.F. Schinazi. Elsevier North Holland Inc.

17. KENNEDY, P.G.E., CLEMENTS, G.B. and BROWN, S.M. (1983). Herpes simplex virus (HSV) infection of cultured foetal cells of neurological origin. Brain 106, 101-119.

18. BROWN, S.M., HARLAND, J. and SUBAK-SHARPE, J.H. (1984). Isolation of restriction endonuclease site deletion mutants of herpes simplex virus. *Journal of General Virology* 65, 1053-1068.

19. LEWIS, M.E., BROWN, S.M., WARREN, K.G. and SUBAK-SHARPE, J.H. (1984). Recovery of herpes simplex virus genetic information from human trigeminal ganglion cells following superinfection with herpes simplex virus type 2 temperature-mutants. *Journal of General Virology* 65, 215-219.

20. HARLAND, J. and BROWN, S.M. (1985). Isolation and characterisation of deletion mutants of herpes simplex virus type 2 (strain HG52). *Journal of General Virology* 66, 1305-1321.

21. COOK, S.D., AITKEN, D.A., LOEFFLER, K.U. and BROWN, S.M. (1986). Herpes simplex virus in the cornea: an ultrastructural study on viral reactivation. *Transactions of the Ophthalmological Societies of the UK* 105, 634-641.

22. BROWN, S.M. and HARLAND, J. (1987). Three mutants of herpes simplex virus type 2: one lacking genes US10, US11 and US12 and two in which R_S has been extended by 6kb to 0.91 map units with loss of U_S sequences between 0.94 and the U_S/TR_S junction. *Journal of General Virology* 68, 1-18.

23. COOK, S.D., AITKEN, D.A. and BROWN, S.M. (1987). Growth and characterisation of rabbit corneal cells in vitro. *Graefe's Archive for Clinical and Experimental Ophthalmology* 225, 351-356.

24. COOK, S.D., BATRA, S. and BROWN, S.M. (1987). Recovery of herpes simplex virus from the corneas of experimentally infected rabbits. *Journal of General Virology* 68, 2013-2017.

25. COOK, S.D. and BROWN, S.M. (1987). Herpes simplex virus type 1 latency in rabbit corneal cells in vitro: reactivation and recombination following intratypic superinfection of long term cultures. *Journal of General Virology* 68, 813-824.

26. MacLEAN, A.R. and BROWN, S.M. (1987). Generation of a herpes simplex virus type 1 (HSV1) variant devoid of Xba1 sites. *Journal of General Virology* 68, 1165-1171.

27. MacLEAN, A.R. and BROWN, S.M. (1987). A herpes simplex virus type 1 variant which fails to synthesise immediate early polypeptide VmwIE63. *Journal of General Virology* 68, 1339-1350.

28. MacLEAN, and BROWN, S.M. (1987). Deletion and duplication variants around the long repeats of herpes simplex virus type 1 strain 17. *Journal of General Virology* 68, 3019-3031.

29. HARLAND, J. and BROWN, S.M. (1988). Generation of a herpes simplex virus type 2 variant devoid of Xba1 sites: removal of the 0.91 map coordinate site results in impaired synthesis of glycoprotein G-2. *Journal of General Virology* 69, 113-124.

30. TAHA, M.Y., BROWN, S.M. and CLEMENTS, G.B. (1988). Neurovirulence of individual plaque stocks of herpes simplex virus type 2 strain HG52. *Archives of Virology* 103, 15-25.

31. BATRA, S.K. and BROWN, S.M. (1989). Analysis of unselected HSV1 McKrae/HSV2 HG52 recombinants demonstrates preferential recombination between intact genomes and restriction endonuclease fragments containing an origin of replication. *Archives of Virology* 105, 1-13.

32. HARLAND, J. and BROWN, S.M. (1989). A herpes simplex virus type 2 variant in which a deletion across the L-S junction is replaced by single or multiple reiterations of extraneous DNA. *Journal of General Virology* 70, 2121-2137.

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35. TAHA, M.Y., CLEMENTS, G.B. and BROWN, S.M. (1989). A herpes simplex virus type 2 strain HG52 variant JH2604 has a 1488bp deletion which eliminates neurovirulence in mice. *Journal of General Virology* 70, 3073-3078.

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